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Date:

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:	Yuqiu Jiang et al.
Group Art Unit:	1635
Application No:	09/604,287
Filed:	June 22, 2000
For:	COMPOSITIONS FOR THE TREATMENT AND DIAGNOSIS OF BREAST CANCER AND METHODS FOR THEIR USE
Examiner:	Janet L. Epps, Ph.D.
Docket No.:	210121.470C7

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DECLARATION OF DR. GARY FANGER

Commissioner for Patents
Washington, D.C. 20231

The undersigned, Dr. Gary Fanger, hereby declares:

1. I am an Associate Scientist at Corixa Corporation, the assignee of the subject application. The following experiments were carried out under my direct supervision.

2. ANALYSIS OF B726P EXPRESSION USING IMMUNOPRECIPITATION AND WESTERN BLOT ANALYSIS

As described in the specification, the polynucleotide sequence provided in SEQ ID NO:474 comprises a splice variant of the B726P gene that brings together into a single ORF both the downstream and upstream ORFS of B726P (see for example page 122, lines 25 – 29). The amino acid sequence encoded by SEQ ID NO:474, the

combined ORF of B726P, is provided in SEQ ID NO:475 Affinity purified polyclonal antibodies generated against the B726P downstream ORF protein set forth in SEQ ID NO:176 (anti-B726Pdown; see page 131, line 12 – page 133, line 10 of the specification) were used to assess the protein expression of the combined ORF of B726P in breast cancer cell lines as compared to normal cells as described below. Since the combined ORF inlcudes both the upstream and downstream ORFs, the antibodies generated against the downstream ORF crossreact with the combined ORF polypeptide as set forth in SEQ ID NO:475.

Cells were lysed in 1% Triton lysis buffer on ice for 10 minutes. Lysates were centrifuge at 15000 rpm and supernatant was saved for immunoprecipitation (IP)/Western analysis. 2 µg of anti-B726down polyclonal antibody was added to the supernatant and rocked overnight at 4°C. 20 µl of protein G bead slurry was added and incubated for 1 hour. Beads were then washed 3 times with 1 ml of lysis buffer. LDS sample buffer and β-mercaptoethanol were added and the samples were heated for 5 min at 95°C. Samples were size fractionated by gel electrophoresis, transferred to nitrocellulose and Western blotted with the mouse anti-B726down monoclonal antibody A2.1.

^{35}S methionine labeling/IP analysis was carried out as follows: Cells were grown in 10% Fetal Bovine Serum (FBS) media to desired density. Cells were then starved with DMEM lacking methionine containing 0.1% FBS media for 10 – 15 minutes. FBS was added to a final concentration of 10% along with ^{35}S-Methionine translabel (300 µCi – 1 mCi). After incubating for 3 –4 hours the cells were harvested, washed, and lysed. B726P was immunoprecipitated as described above and samples were size fractionated by gel electrophoresis before being exposed to autoradiography film.

The results from the above described experiments show that the full length 148 kDa form (also called NYBR1), the 110 kDa combined ORF form, and the 35 kDa downstream ORF form are all expressed in breast tumor cell lines HTB21 and BT474 but not in the SKBR3 normal breast cell line. Therefore, these results confirm that the combined ORF is an expressed protein that is found in breast tumor cell lines and not in

normal cells.

3. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Gary Fanger, Ph.D. 4/15/02
Date